

In the claims:

1. (Original) A method of generating a high density cell culture, the method comprising:
  - a) placing cells and a medium in a simple culture vessel, thereby generating a cell culture, the medium being suitable for growth of a high density cell culture; and
  - b) operating the simple culture vessel to provide an oxygen transfer rate suitable for growth of a high density cell culture, thereby generating a high density cell culture, the high density cell culture having an optical density at a wavelength of 600 nm ( $OD_{600}$ ) of 4 or greater.
2. (Original) The method of claim 1, wherein the oxygen transfer rate is greater than 2.0 millimoles  $O_2$ /liter/minute.
3. (Original) The method of claim 1, wherein the cells are bacterial cells.
4. (Original) The method of claim 3, wherein a substantial portion of the bacterial cells comprise an exogenously regulated expression construct.
5. (Original) The method of claim 4, further comprising contacting the cell culture with an inducer.
6. (Original) The method of claim 4, wherein the exogenously regulated expression construct comprises a lacI binding site operably linked to an expressible nucleic acid, and wherein the inducer is IPTG.

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7. (Original) The method of claim 5, wherein the cell culture is contacted with the inducer when the culture has an OD<sub>600</sub> of 1 or greater.
8. (Original) The method of claim 5, wherein the cell culture is substantially maintained at a temperature higher than 25°C prior to contacting the cell culture with the inducer and the cell culture is substantially maintained at a temperature lower than 25°C after contacting the cell culture with the inducer.
9. (Original) The method of claim 1, wherein the simple culture vessel is shaken on an orbital shaker table with an orbital diameter and shaking rate (revolutions per minute) suitable to provide an oxygen transfer rate greater than 2.0 millimoles O<sub>2</sub>/liter/minute.
10. (Original) The method of claim 9, wherein the orbital shaker table has an orbital diameter of at least about one inch and the shaking rate is at least about 200 revolutions per minute.
11. (Original) The method of claim 1, wherein the simple culture vessel is shaken at greater than 250 revolutions per minute.
12. (Original) The method of claim 11, wherein the cell culture has a volume of less than 200 milliliters.
13. (Original) The method of claim 1, wherein the optical density at a wavelength of 600 nm is 10 or greater.

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14. (Original) The method of claim 13, wherein the cell culture has a volume of between 500 and 2000 milliliters.

15. (Original) The method of claim 1, wherein the optical density at a wavelength of 600 nm is 20 or greater.

16. (Original) The method of claim 15, wherein the cell culture has a volume of less than 200 milliliters.

17. (Original) The method of claim 1, wherein the medium comprises:

i) a carbon source selected from the group consisting of glycerol and glucose;

ii) a complex organic material selected from the group consisting of tryptone, yeast extract, hydrolyzed casein and beef broth; and

iii) a magnesium source.

18. (Original) The method of claim 17, the medium further comprising:

iv) two or more metals selected from the group consisting of cobalt, manganese, copper, boron, molybdenum, zinc, iron, calcium, aluminum and nickel.

19. (Original) The method of claim 1, wherein the medium comprises:

i) tryptone at a concentration of 10 – 14 grams/L;

- ii) yeast extract at a concentration of 20 – 30 grams/L;
- iii) buffering salts at an initial pH of between 6 and 8;
- iv) magnesium sulfate at a concentration of between 0.5 and 2 mM;
- v) 0.5 – 5% glycerol; and
- vi) a metal mixture comprising cobalt, manganese, copper, boron, molybdenum, zinc, iron, calcium, aluminum and nickel.

20. (Original) The method of claim 19, wherein the buffering salts comprise potassium and phosphate, the phosphate at a concentration of between 100 and 200 mM.

21. (Original) The method of claim 19, wherein the metal mixture comprises:  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  (4 – 8  $\mu\text{M}$ ),  $\text{MnSO}_4 \cdot 5\text{H}_2\text{O}$  (20 – 40  $\mu\text{M}$ ),  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  (4 – 8  $\mu\text{M}$ ),  $\text{H}_3\text{BO}_3$  (6 – 10  $\mu\text{M}$ ),  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$  (6 – 10  $\mu\text{M}$ ),  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  (4 – 8  $\mu\text{M}$ ),  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  (75 - 125  $\mu\text{M}$ ),  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  (40 - 100  $\mu\text{M}$ ),  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  (2 - 6  $\mu\text{M}$ ), and  $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$  (6 - 10  $\mu\text{M}$ ).

22. (Original) The method of claim 1, further comprising obtaining a partially purified polypeptide composition from the high density cell culture.

23. (Original) The method of claim 1, further comprising obtaining a purified polypeptide from the high density cell culture.

24. (Original) The method of claim 23, the method further comprising determining an NMR structure of the polypeptide.

25. (Original) The method of claim 23, the method further comprising determining an X-ray crystal structure of the polypeptide.
26. (Original) The method of claim 23, the method further comprising determining a druggable region of the polypeptide.
27. (Original) The method of claim 1, wherein the simple culture vessel is a Tunac-type flask.
28. (Original) The method of claim 1, wherein the simple culture vessel is a 96-well plate.
29. (Original) A high density cell culture produced according to the method of claim 1.
30. (Original) A partially purified polypeptide composition produced according to the method of claim 22.
31. (Original) A purified polypeptide composition produced according to the method of claim 23.
32. (Original) The purified polypeptide composition of claim 31, wherein the polypeptide composition comprises a polypeptide selected from the group consisting of: a therapeutically useful polypeptide, an industrially useful polypeptide and a polypeptide useful for research purposes.
33. (Original) The method of claim 1, further comprising contacting the cell culture with a label that may be incorporated into a polypeptide during growth of the high density cell culture.

34. (Original) The method of claim 33, wherein the label is seleno-L-methionine.

35. (Original) The method of claim 33, wherein the label is an isotopic label selected from the group consisting of potassium-40 ( $^{40}\text{K}$ ), carbon-14 ( $^{14}\text{C}$ ), tritium ( $^3\text{H}$ ), sulphur-35 ( $^{35}\text{S}$ ), phosphorus-32 ( $^{32}\text{P}$ ), technetium-99m ( $^{99\text{m}}\text{Tc}$ ), thallium-201 ( $^{201}\text{Tl}$ ), gallium-67 ( $^{67}\text{Ga}$ ), indium-111 ( $^{111}\text{In}$ ), iodine-123 ( $^{123}\text{I}$ ), iodine-131 ( $^{131}\text{I}$ ), yttrium-90 ( $^{90}\text{Y}$ ), samarium-153 ( $^{153}\text{Sm}$ ), rhenium-186 ( $^{186}\text{Re}$ ), rhenium-188 ( $^{188}\text{Re}$ ), dysprosium-165 ( $^{165}\text{Dy}$ ), holmium-166 ( $^{166}\text{Ho}$ ), hydrogen-1 ( $^1\text{H}$ ), hydrogen-2 ( $^2\text{H}$ ), hydrogen-3 ( $^3\text{H}$ ), phosphorous-31 ( $^{31}\text{P}$ ), sodium-23 ( $^{23}\text{Na}$ ), nitrogen-14 ( $^{14}\text{N}$ ), nitrogen-15 ( $^{15}\text{N}$ ), carbon-13 ( $^{13}\text{C}$ ) and fluorine-19 ( $^{19}\text{F}$ ).

36. (Original) The method of claim 33, wherein the label is a heavy atom label selected from the group consisting of cobalt, selenium, krypton, bromine, strontium, molybdenum, ruthenium, rhodium, palladium, silver, cadmium, tin, iodine, xenon, barium, lanthanum, cerium, praseodymium, neodymium, samarium, europium, gadolinium, terbium, dysprosium, holmium, erbium, thulium, ytterbium, lutetium, tantalum, tungsten, rhenium, osmium, iridium, platinum, gold, mercury, thallium, lead, thorium and uranium.

37. (Original) The method of claim 1, wherein the medium comprises:

- i) a carbon source selected from the group consisting of glycerol and glucose;
- ii) a basal nutrient source; and
- iii) a label selected from the group consisting of an isotopic label, a heavy atom label, and seleno-L-methionine.

38. (Original) The method of claim 37, the medium further comprising one or more amino acids.

39. (Original) The method of claim 37, wherein the medium comprises: 0.04 - 4% glucose, NH<sub>4</sub>Cl at a concentration of 20-60 mM, KH<sub>2</sub>PO<sub>4</sub> at a concentration of 20-60 mM, Na<sub>2</sub>HPO<sub>4</sub> at a concentration of 75-115 mM, Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O at a concentration of 75-115 mM, MgSO<sub>4</sub> at a concentration of 0.5-4 mM, FeSO<sub>4</sub> at a concentration of 70-110 mM, CaCl<sub>2</sub> at a concentration of 80-120 µM, one or more amino acids but not methionine, vitamins, and seleno-L-methionine at a concentration of 100-300 µM.

40. (Original) A method of culturing cells, the method comprising:

a) placing cells and a medium in a simple culture vessel, thereby generating a cell culture, the medium comprising:

- i) tryptone at a concentration of 10 – 14 grams/L;
- ii) yeast extract at a concentration of 20 – 30 grams/L;
- iii) buffering salts at a pH of between 6 and 8; and
- iv) MgSO<sub>4</sub> at a concentration of between 0.5 and 2 mM; and

b) operating the simple culture vessel to provide an oxygen transfer rate greater than 2.0 millimoles O<sub>2</sub>/liter/min.

41. (Original) The method of claim 40, wherein the medium further comprises:

- v) 0.5 – 5% glycerol;
- vi) a metal mixture comprising: cobalt, manganese, copper, boron, molybdenum, zinc, iron, calcium, aluminum and nickel; and
- vii) vitamins.

42. (Original) The method of claim 41, wherein the metal mixture comprises: CoCl<sub>2</sub>-6H<sub>2</sub>O (4 – 8 µM), MnSO<sub>4</sub>-5H<sub>2</sub>O (20 – 40 µM), CuCl<sub>2</sub>-2H<sub>2</sub>O (4 – 8 µM), H<sub>3</sub>BO<sub>3</sub> (6 – 10 µM), Na<sub>2</sub>MoO<sub>4</sub>-2H<sub>2</sub>O (6 – 10 µM), ZnSO<sub>4</sub>-7H<sub>2</sub>O (4 – 8 µM), FeSO<sub>4</sub>-7H<sub>2</sub>O (75 - 125 µM), CaCl<sub>2</sub>-2H<sub>2</sub>O (40 - 100 µM), AlCl<sub>3</sub>-6H<sub>2</sub>O (2 - 6 µM), and NiCl<sub>2</sub>-6H<sub>2</sub>O (6 - 10 µM).

43. (Original) The method of claim 40, wherein the simple culture vessel is a Tunac-type flask.

44. (Original) A method for producing a labeled polypeptide, the method comprising:

- a) placing cells and a medium in a simple culture vessel, thereby generating a cell culture, the medium comprising:
  - i) a carbon source selected from the group consisting of glycerol and glucose;
  - ii) a basal nutrient source; and
  - iii) a label selected from the group consisting of an isotopic label, a heavy atom label, and seleno-L-methionine; and

- b) operating the simple culture vessel to provide an oxygen transfer rate greater than 2.0 millimoles O<sub>2</sub>/liter/min.

45. (Original) The method of claim 44, wherein the medium comprises: 0.04 - 4% glucose, NH<sub>4</sub>Cl at a concentration of 20-60 mM, KH<sub>2</sub>PO<sub>4</sub> at a concentration of 20-60 mM, Na<sub>2</sub>HPO<sub>4</sub> at a concentration of 75-115 mM, Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O at a concentration of 75-115 mM, MgSO<sub>4</sub> at a concentration of 0.5-4 mM, FeSO<sub>4</sub> at a concentration of 70-110 mM, CaCl<sub>2</sub> at a concentration of 80-120 µM, one or more amino acids but not methionine, vitamins, and seleno-L-methionine at a concentration of 100-300 µM.

46. (Original) The method of claim 44, wherein the simple culture vessel is a Tunac-type flask.

47. (Original) A method for high-throughput production of polypeptides, the method comprising:

- a) obtaining a plurality of cell lines, each cell line comprising an exogenously controlled expression construct for expressing a nucleic acid encoding a polypeptide;
- b) generating a plurality of cell cultures by placing in separate simple culture vessels
  - i) medium suitable for growth of a high density cell culture, and
  - ii) cells of one of the plurality of cell lines; and

- c) operating the simple culture vessels to provide an oxygen transfer rate suitable for growth of a high density cell culture, thereby obtaining a high density cell culture having an OD<sub>600</sub> of 4 or greater; and
- d) obtaining a purified polypeptide composition from a plurality of the high density cell cultures, thereby obtaining purified polypeptide compositions.

48. (Original) The method of claim 47, wherein each cell line comprises an exogenously controlled expression construct for expressing a nucleic acid encoding a polypeptide, wherein each polypeptide is a polypeptide of the proteome of a subject organism.

49. (Original) The method of claim 47, wherein each cell culture is contacted with an inducer at an OD<sub>600</sub> of 1 or greater.

50. (Original) The method of claim 47, wherein each cell culture is contacted with inducer at approximately the same time after generating the cell culture.

51. (Original) A cell culture medium comprising:

- a) tryptone at a concentration of 10 – 14 grams/L;
- b) yeast extract at a concentration of 20 – 30 grams/L;
- c) buffering salts at an initial pH between 6 and 8;
- d) a metal mixture comprising: cobalt, manganese, copper, boron, molybdenum, zinc, iron, calcium, aluminum and nickel; and

e) a magnesium source.

52. (Original) The cell culture medium of claim 51, wherein the magnesium source is MgSO<sub>4</sub>.

53. (Original) The cell culture medium of claim 51, the medium further comprising 0.5 – 5% glycerol.

54. (Original) The cell culture medium of claim 51, wherein the metal mixture comprises: CoCl<sub>2</sub>-6H<sub>2</sub>O (4 – 8 µM), MnSO<sub>4</sub>-5H<sub>2</sub>O (20 – 40 µM), CuCl<sub>2</sub>-2H<sub>2</sub>O (4 – 8 µM), H<sub>3</sub>BO<sub>3</sub> (6 – 10 µM), Na<sub>2</sub>MoO<sub>4</sub>-2H<sub>2</sub>O (6 – 10 µM), ZnSO<sub>4</sub>-7H<sub>2</sub>O (4 – 8 µM), FeSO<sub>4</sub>-7H<sub>2</sub>O (75 – 125 µM), CaCl<sub>2</sub>-2H<sub>2</sub>O (40 - 100 µM), AlCl<sub>3</sub>-6H<sub>2</sub>O (2 - 6 µM), and NiCl<sub>2</sub>-6H<sub>2</sub>O (6 - 10 µM).

55. (Original) The cell culture medium of claim 51, wherein the buffering salts comprise potassium and phosphate, the phosphate at a concentration of between 100 and 200 mM.

56. (Original) A dry composition that, when mixed with an appropriate volume of water, provides a cell culture medium of any of claim 51 - 55.

57. (Original) A concentrated medium that, when mixed with an appropriate volume of water, provides a cell culture medium of any of claim 51 – 55.

58. (Original) A metal mixture comprising water and at least eight elements selected from the group consisting of: cobalt, manganese, copper, boron, molybdenum, zinc, iron, calcium, aluminum and nickel.

59. (Original) A metal mixture of claim 58 having a pH between 6 and 8.
60. (Original) A metal mixture of claim 58, comprising cobalt, manganese, copper, boron, molybdenum, zinc, iron, calcium, aluminum and nickel.
61. (Original) The metal mixture of claim 58, comprising: CoCl<sub>2</sub>-6H<sub>2</sub>O (4 – 8 µM), MnSO<sub>4</sub>-5H<sub>2</sub>O (20 – 40 µM), CuCl<sub>2</sub>-2H<sub>2</sub>O (4 – 8 µM), H<sub>3</sub>BO<sub>3</sub> (6 – 10 µM), Na<sub>2</sub>MoO<sub>4</sub>-2H<sub>2</sub>O (6 – 10 µM), ZnSO<sub>4</sub>-7H<sub>2</sub>O (4 – 8 µM), FeSO<sub>4</sub>-7H<sub>2</sub>O (75 - 125 µM), CaCl<sub>2</sub>-2H<sub>2</sub>O (40 - 100 µM), AlCl<sub>3</sub>-6H<sub>2</sub>O (2 - 6 µM), and NiCl<sub>2</sub>-6H<sub>2</sub>O (6 - 10 µM).
62. (Original) A dry composition that, when mixed with an appropriate volume of water, provides a metal mixture of any of claims 58 – 61.
63. (Original) A concentrated metal mixture that, when mixed with an appropriate volume of water, provides a metal mixture of any of claims 58 – 61.